Natural Resource Conservation and Development.

Level - II

Learning Guide #53

Unit of Competence: Facilitate in Performing Ex-Situ Conservation Measures

Module Title: Facilitating in Performing Ex-Situ Conservation Measures

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LO2. Collect, Store and Propagate Planting Materials

Instruction Sheet	Learning Guide #53

This learning guide is developed to provide you the necessary information regarding the following **content coverage** and topics –

- Occupational health and safety
- Gathering and organizing tools and equipment's
- Collecting and storing Prioritized species
- Propagating collected planting materials

This guide will also assist you to attain the learning outcome stated in the cover page. Specifically, upon completion of this Learning Guide, **you will be able to –**

- Observe and follow organizational ***OHS*** procedures, practices, policies, and precautions.
- gather and organize ***tools and equipment*** and, necessary materials are according to forest conservation procedures
- collect and store the Prioritized species are according to forest conservation procedures
- Collect ***planting materials*** for further planting out according to forest conservation procedures

Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described in number 3 to 6.
3. Read the information written in the "Information Sheets 1, Sheets 2, Sheets 3, and Sheets 4".
4. Accomplish the "Self-check 1, Self-check 2, Self-check 3, and Self-check 4" **in page – 4, 7, 12, and 16** respectively.
5. If you earned a satisfactory evaluation from the "Self-check" proceed to "Operation Sheet 1, and Operation Sheet 2" **in page -17 and 19**
6. Do the "LAP test" **in page – 18 and 19** (if you are ready).

Information Sheet-1	Occupational Health and Safety

2.1. Occupational health and safety

Introduction

Occupational health is a multidisciplinary activity aimed at:

- the protection and promotion of the health of workers by preventing and controlling occupational diseases and accidents and by eliminating occupational factors and conditions hazardous to health and safety at work;
- the development and promotion of healthy and safe work, work environments and work organizations;
- the enhancement of the physical, mental and social well-being of workers and support for the development and maintenance of their working capacity, as well as professional and social development at work;
- enabling workers to conduct socially and economically productive lives and to contribute positively to sustainable development.

Occupational health has gradually developed from a mono-disciplinary, risk-oriented activity to a multi-disciplinary and comprehensive approach that considers an individual's physical, mental and social well-being, general health and personal development.

Occupational Health and Safety (**OHS**), also commonly referred to as **occupational** safety and health (OSH), occupational health, or workplace health and safety (WHS), is a multidisciplinary field concerned with the safety, health, and welfare of people at work.

The goals of occupational safety and health programs include to foster a safe and healthy work environment. OHS may also protect co-workers, family members, employers, customers, and many others who might be affected by the workplace environment.

In the United States, the term occupational health and safety is referred to as occupational health and occupational and non-occupational safety and includes safety for activities outside of work.

In common-law jurisdictions, employers have a common law duty to take reasonable care of the safety of their employees. Statute law may in addition impose other general duties, introduce specific duties, and create government bodies with powers to regulate workplace safety issues: details of this vary from jurisdiction to jurisdiction.

Agriculture

Agriculture workers are often at risk of work-related injuries, lung disease, noise-induced hearing loss, skin disease, as well as certain cancers related to chemical use or prolonged sun exposure. On industrialized farms, injuries frequently involve the use of agricultural machinery. The most common cause of fatal agricultural injuries in the United States is tractor rollovers, which can be prevented by the use of roll over protection structures which limit the risk of injury in case a tractor rolls over.

Pesticides and other chemicals used in farming can also be hazardous to worker health and workers exposed to pesticides may experience illnesses or birth defects.

As an industry in which families, including children, commonly work alongside their families, agriculture is a common source of occupational injuries and illnesses among younger workers. Common causes of fatal injuries among young farm worker include drowning, machinery and motor vehicle-related accidents.

The 2010 NHIS-OHS found elevated prevalence rates of several occupational exposures in the agriculture, forestry, and fishing sector which may negatively impact health. These workers often worked long hours.

OHS - May include, Caution is made for every activity that are undertaken during the launching of forest conservation and protection activity.

1. Personal care

light-weight tents with sealed ground sheet

If collecting in areas with no accommodation

- ❖ High quality water filter or purifier system
- ❖ Mosquito net
- ❖ electric torch and lamp with spare batteries
- ❖ Sleeping bags, pillow and blanket
- ❖ matches
- ❖ cooking equipment and stove
- ❖ candles
- ❖ Eating utensils
- ❖ tarpaulin
- ❖ Large and small water containers

Medicines

- anti-malarial pills
- insecticide sprays or repellent creams
- first-aid kit
- pain-killer pills
- snake bite kit for commonly found poisonous snakes
- antipyretics (paracetamol or aspirin)
- antacid tablets
- antiseptic cream or liquid
- anti-diarrhea pills

Transport

- a four-wheel drive motor vehicle with roof rack
- Puncture repair kit
- One set of spare parts and tools
- two Jerri cans for diesel/petrol
- Two spare tires
- engine driven winch and chain or nylon rope
- Pump and pressure gauge

Clothing

- Drip-dry clothes that can be layered for warmth and protection
- Strong high boots for snake infested areas
- Lightweight jackets and long sleeved shirts with plenty of pockets
- Sweater and water proof clothing if collecting during rainy season.

Self-Check – 1	**Written Test**

Name: _____ **Date:** _____

Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page.

1. Define Occupational health and safety? (2pts.)
2. What personal care would consider during collecting material? (3pts)

Note: **Satisfactory rating - 3 points** **Unsatisfactory - below 3 points**

Answer Sheet

Score = _____

Rating: _____

Information Sheet-2	Gathering and organizing tools and equipment’s

2.2. Gathering and organizing tools and equipment’s

The basic equipment needed for collection is as follows:

1. Harvesting tools

- Cloth bags or nylon net bags
- drying stove and stand
- Seed envelopes
- Packing sacks
- Strong knife or secateurs
- Digger and sieves for sifting soil (important for collection of groundnut and its wild species)
- Scissors
- Pencil, pens and permanent markers of different colors
- Field or collector’s notebooks
- Stapler and staples
- Rubber bands for closing bags
- pocket knife
- knives, trowels,
- spades, forks, rakes, hoes, shovels,
- buckets, brooms,
- wheelbarrows, hoses and hose fittings, tree-planters, secateurs or snips, and machinery such as tractors,
- Canvas, safety helmet, bags,
- refrigerator, weight, ladder, car, cold storage clean, containers for holding seeds, vacuum seed collecting machines,

In line with the principle of national sovereignty over plant genetic resources, and Article 7 of International Code of Conduct for Plant Germplasm Collecting and Transfer, germplasm collectors should secure from relevant authorized government body:

- Prior approval for collecting,
- Material transfer agreements for transfer of the collected material incorporating provisions for further handling, storage, regeneration, utilization and distribution
- Labels (preferably tear-off tags) for labeling specimens
- Gloves
- receipt pad
- Plant press with corrugated aluminum sheets
- Local currency in small denomination
- Absorbent paper for pressing specimen

2. Scientific equipment

- Portable altimeter
- Light meter
- Geographical Positioning System (GPS)
- Pocket lenses
- Field compass for emergency use
- Soil sample kit
- Cameras with close-up lenses and filters
- Binoculars
- Digital camera with charger
- Calendar

3. Printed material

- ⇒ Regional flora
- ⇒ printed slips with institute's address
- ⇒ Road maps
- ⇒ collection data sheets
- ⇒ Vegetation or climate data
- ⇒ herbarium and quarantine labels
- ⇒ List of rest houses or hotels
- ⇒ visiting cards
- ⇒ Import Permit or other required permits

Self-Check – 2	Written Test

Name: _____ **Date:** _____

Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page.

1. List at least four scientific equipment? (4pts)
2. write down at least four harvesting tools used for collection? (4pts)

Note: **Satisfactory rating - 4 points** **Unsatisfactory - below 4 points**

Answer Sheet

Score = _____

Rating: _____

Information Sheet-3	Collecting and storing Prioritized species

2.3. Collecting and storing Prioritized species

Ex situ conservation of germplasm includes a series of activities that begin with the acquisition of material and can even include the use of this material or its loan for use. The activities, or stages, include:

- Acquiring germplasm
- Multiplying before storage
- Storing
- Managing the conserved germplasm.

  This includes:

  - ✓ Characterization and evaluation
  - ✓ Multiplication and/or regeneration for distribution and use
  - ✓ Documentation
  - ✓ Use or loan for use

A `germplasm` is a collection of genetic resources for an organism. For plants, the germplasm may be stored as a seed collection or, for trees, in a nursery.

"**Genetic material**" means any material of plant origin, including reproductive and vegetative propagating material, containing functional units of heredity.

The main reasons for collecting germplasm are:

- Genetic erosion — loss of genetic diversity.
- Gap filling — when diversity is missing or insufficiently represented in an existing collection.
- Need based — for breeding, research, or developmental work

Collecting germplasm is expensive. Therefore, make a critical review of the past collection activities of the crop before embarking on a collection trip.

If germplasm was already collected from the area, correspond with the collector(s) and obtain duplicate samples.

Methods of collection

Germplasm collections are congregations of accessions that represent the genetic variability targeted for conservation and/or use. They can hold from tens of samples to thousands, all maintained under the appropriate environments and conditions. Germplasm collections are classified according to base, active, core and working collections. Each is composed of certain materials, conserved under particular conditions and kept for specific periods.

A. Base collection

A base collection groups the possible genetic variability of the species of interest, including wild relatives, intermediate forms, cultivars, traditional varieties and elite germplasm.

The collection is established to conserve germplasm on a long-term basis and to recover missing accessions. It is not used for distribution or exchange. It may contain samples of seed (orthodox only) or vegetative material. If it contains seeds, the moisture content of these is reduced to 3% – 7%. The seeds are then packed in sealed containers and stored in chambers at temperatures between -10°C and -20°C. If vegetative material is conserved, it is maintained in the field or is cryopreserved.

B. Active collection

An active collection is a duplicate of a base collection, established for the short and medium terms for management and distribution. It can conserve germplasm in the form of seed, in the field or in vitro. If it conserves seeds, these are stored at a moisture content between 3% and 7% and at temperatures between 0°C and 15°C. If the material is conserved in vitro, it is conserved under slow growth.

Active collections can be the responsibility of a variety of institutions, both public and private, and including IARCs; regional, national, provincial and municipal programs; universities; and non-governmental organizations.

C. Core collection

The core collection brings together the greater genetic variability of a species with the least number of samples. It is formed by duplicating the base collection, separating those accessions that will constitute the core collection – that is, 70% to 80% of variability will be represented by 10% to 15% of the accessions – and taking the rest to a reserve collection.

The core collection is established to facilitate germplasm management and promote its use. It permits the detection of duplicates in the base collection and helps establish priorities for characterizing and evaluating samples. It also offers easy access to conserved materials.

D. Working collection

A working collection, or breeding collection, is established to provide germplasm to researchers, institutions or research and/or breeding programs. It holds accessions with traits of interest for crop improvement, but is unrepresentative of a crop's genetic variability. It conserves seeds or plants over the short term. Seeds are kept at room temperature but if the climate is hot and moist they are put in rooms fitted with air conditioners and dehumidifiers. Plants are conserved in the field or in glasshouses. Working collections are normally the responsibility of crop breeding programs.

Be inquisitive to acquire information on anything interesting;

- Collectors' and collection number
- Date and site of collection
- Geographical coordinates of collection sites
- Status of sample (wild, weedy, cultivated)
- Source of collection (field, market sample or farm store)
- Label the collection bags both within and outside

Storing planting materials

Planting materials - are materials used for propagation to establish a plantation

1.Seed Banking

Undeniably, the most cost-effective method of providing plant genetic resources for long-term *ex situ* conservation is through the storage of seeds under very specific conditions, following techniques well developed for crop plants by organizations such as the International Plant Genetic Resources Institute (IPGRI), previously the International Board of Plant Genetic Resources (IBPGR) and the Food and Agricultural Organization of the United Nations (FAO). The main advantage of seed banking is that it allows large populations to be preserved and genetic erosion to be minimized by providing optimum conditions and reducing the need for regeneration. However, when a natural population still exists, it may be advisable to re-collect rather than regenerate a new supply from the previous collection as damage can occur such as mutations associated with the loss of viability during storage.

2. Field Gene banks

Field gene banks or living collections are the main conservation strategy for long-lived perennials, recalcitrant species and vegetative propagated species. Their main limitation is that they take a great deal of space and are difficult to maintain and protect from natural disasters. They are susceptible to the spread of diseases and may suffer from neglect.

Furthermore, out-breeders require controlled pollination for regeneration from seed. In many circumstances they are the only available option for the conservation of important germplasm. When displayed, the plants have an important educational value and can easily be accessed for research purposes.

3. In vitro Storage Methods

The storage of germplasm in laboratory conditions (*in vitro*) is especially suited for the long-term conservation of recalcitrant species and vegetative propagated species. They can be stored at low temperature under slow growth conditions or cryopreserved in liquid nitrogen at -196ºC. Cryopreservation has so far been successful with only a relatively few species but is a very promising development for long-term storage (WCMC, 1992). The main limitation of in vitro storage is the need for special equipment, techniques and trained staff.

4. Pollen Banks

Pollen preservation may be useful for base collections of species that do not produce orthodox seeds. It requires little space but some cytoplasmic genes would be lost. Like seeds, pollen can be divided into desiccation tolerant and intolerant. However, information about storage characteristics of pollen from wild species is fragmentary, existing mainly for some crop relatives and for medicinal and forest species

Self-Check – 3	Multiple Choice

Name: _____ **Date:** _____

Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page.

I. Choose the correct answer from a given alternative (3pt each)

1. _____ groups the possible genetic variability of the species of interest, including wild relatives, intermediate forms, cultivars, traditional varieties and elite germplasm.

A) Base collection. C) Core collection.

B) C) Working collection. D) Active collection

2. Which collection method brings together the greater genetic variability of a species with the least number of samples?

A). base collection. B) Core collection.

C) Working collection D) Active collection

3. Which one of the following is **not** the main reasons for collecting germplasm

A. To maintain genetic erosion. B. Gap filling

C. For breeding D. None

4. _____are the main conservation strategy for long-lived perennials, recalcitrant species and vegetative propagated species.

A. Field gene banks C. In vitro

B. Seed gene bank D. Pollen bank.

Note: **Satisfactory rating - 6 points** **Unsatisfactory - below 6 points**

Answer Sheet

Score = _____

Rating: _____

Information Sheet-4	Propagating collected planting materials

2.4. Propagating collected planting materials

There are two methods of propagation planting stocks. These are:

- ***Seed (or sexual) propagation***
- ***Clonal (or asexual/vegetative) propagation***

The concept of vegetative propagation is that an ***exact copy of the genome*** of a mother plant is made and continued in new individuals. This is possible because plants, - unlike animals or humans, - have meristematic, undifferentiated cells that can differentiate to the various organs necessary to form a whole new plant. A piece of plant shoot, root, or leaf, can therefore, grow to form a new plant that contains the exact genetic information of its source plant.

Whereas sexual reproduction by seeds provides opportunity for variation and evolutionary advancement, vegetative propagation aims at the identical reproduction of plants with desirable features such as high productivity, superior quality, or high tolerance to biotic and/or abiotic stresses, and as such, plays a very important role in continuing preferred trait from one generation to the next.

After identification of germplasm, the elite plant material must be collected and conserved. Characterization, evaluation and maintenance of the germplasm are very important, for loss of unique genotypes represents a loss of genetic diversity. Suitable methods of propagation must be available if the material is to be preserved. in vitro propagation is one such technique, and is especially useful for the production of virus-free plants for germplasm repositories, rapid propagation of selected genotypes in breeding programs, propagation of plants for which conventional methods are not available and also for large-scale multiplication of new cultivars. Tissue culture is also used for the international exchange of plant materials. These issues will be addressed with particular reference to temperate zone fruits in the tropics and subtropics (TZFTS). Clean, pest-free plantlets essential to many research projects We will develop new protocol for in vitro propagation of some plant species.

Cryopreservation is a process where cells or whole tissues are preserved by cooling too low sub-zero temperatures, such as (typically) 77 K or −196 °C (the boiling point of liquid nitrogen).

At these low temperatures, any biological activity, including the biochemical reactions that would lead to cell death, is effectively stopped. However, when cry protectant solutions are not used, the cells being preserved are often damaged due to freezing during the approach to low temperatures or warming to room temperature.

Vegetative propagation

The use of vegetative propagation is based on the 'tot potency' of living cells in plant, i.e. capacity of a tissue to regenerate to a whole and identical plant as the mother tree. Therefore, clones produced from the same mother plant are genetically identical, unless rare somatic mutations occur.

Vegetative propagation is the key to preserving genetic resources of high value species threatened by extinction in the wild, obtain uniform tree products, and accelerating fruit production by reducing the time to fruiting compared to trees produced from seed regeneration.

The collected plant material is washed and disinfected before propagation and transport to the place of conservation. Disinfection can be done with bactericides, fungicides (for bulbs and rhizomes) or thermotherapy (for stakes).

Once disinfected, the vegetative material is propagated in the field, glasshouse or *in vitro*. In the field and in glasshouses, samples are planted in seedbeds or in pots and left to grow until mature enough to permit the taking of new samples. The procedure is repeated until the necessary number of plants is obtained for establishing the collection in the definitive site.

If propagation is to be *in vitro*, samples are planted in glasshouses, in soils of optimal nutritional quality. From the resulting plants – preferably the youngest – explants are extracted for micro propagation *in vitro* until complete plants are obtained which are then taken to the glasshouse, where they are planted in sterile soil. Two to three weeks later, they are transferred to the definitive site in the field. Micro propagation consists of:

- disinfecting explants in a solution of sodium hypochlorite or calcium, chloride of mercury or ethanol,

- culturing the explants in an *in vitro* culture medium until they produce new shoots, and
- rooting the shoots until entire plants are obtained

Propagation in the field and in glasshouses is simple but requires time and space and does not guarantee that the plants obtained are healthy and genetically identical to the originals. *In vitro* propagation solves these problems and makes the propagation of many species possible, including those that reproduce by seed, as it is more convenient.

The most important vegetative propagation techniques for tree species are the propagation by cuttings, layering, budding, grafting and micro propagation. The most important reasons for vegetative propagation are:

- Maintaining superior genotypes
- Problematic seed germination and storage
- Shortening time to flower and fruit
- Combining desirable characteristics of more than one genotype into single plant
- Controlling phases of development
- Uniformity of plantations.

1. Cuttings

Soft current growth tissue or slightly woody older material.10-30cm long and 0.3-2.0cm in diameter. Usually one or two leaves are left at the tip. The cutting is carefully sown into the growing medium.

Advantages:

⇒ Rapid tree improvement- use best genetic material.

Disadvantages:

⇒ Need young juvenile material e.g. young shoots from the base

⇒ Need precise environmental conditions for some species

⇒ Careful management is required in planting out.

2. Micro-propagation (tissue culture)

Not currently relevant to Dry land Ethiopia except perhaps for high-value fruit tree production.

3. Grafting

This is the method of propagation where a vegetative part is removed from the parent tree (the scion) and is attached to a part with its own toot (the stock) so that the tissues fuse.

Advantages:

- Rapid tree improvement- use best genetic material.

Disadvantages:

- Need young juvenile material.
- Need precise environmental
- Careful management is required

Self-Check – 4	**Written Test**

Name: _____ **Date:** _____

Directions: Answer all the questions listed below. Use the Answer sheet provided in the next page.

1. what is Cryopreservation? (3pts)
2. Write down methods of vegetative propagation? (3pts)
3. what is most important reason for vegetative propagation? (4pts)

Note: **Satisfactory rating - 5 points** **Unsatisfactory - below 5 points**

Answer Sheet

Score = _____

Rating: _____

Operation Sheet 1	Collecting and storing Prioritized species

Method of Collecting and storing Prioritized species

Step 1- Gather Information about finical, possibility and permission before collection of planting material

Step 2- Processing and conservation arrangements for planting material samples

Step 3- Respect local customs, traditions, and values, and property rights

Step 4. Identify planting materials (endangered species)

Step 5. Choose appropriate collection techniques based on your objective

Step 6. Under take collection from identified material

Step 7. Deposit duplicate sets of all collections and associated materials and records of any pertinent information

Step 8. Make arrangements with quarantine officials, seed storage managers and curators to ensure that the samples are transferred as quickly as possible to conditions which optimize their viability.

Step 9. Store germplasm in accordance with the requirements

Step 10. prepare a consolidated report

LAP Test	Practical Demonstration

Name_____ Date: _____

Time started: _____ Time finished: _____

Instructions: Given necessary templates, tools and materials you are required to perform the following tasks within ------hour.

Task 1. Collect and Store planting materials

Operation Sheet 2	Propagating collected planting materials

Method of Propagating collected planting materials

Step 1- Select mother tree for cuttings.

Step 2. Decide the type of cutting (leaf, root, or stem).

Step 3. Trim leaves before the shoots are detached from the stock plants.

Step 4. Treat cuttings with a pesticide or soaked in a surface sterility.

Step 5. Use a polyethylene bag that is moistened inside to carry the cuttings.

Step 6. Keep the collected shoots under shade, without throwing or squeezing the bags

LAP Test	Practical Demonstration

Name_____ Date: _____

Time started: _____ Time finished: _____

Instructions: Given necessary templates, tools and materials you are required to perform the following tasks within ------hour.

Task 1. Propagate collected planting material

List of Reference Materials

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4. Wang, B.S.P., P. Charest, and B. Downie. 1993. *Ex situ* storage of seeds, pollen and *in vitro* perennial woody plant species.
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